

Low-energy shock wave treatment induces angiogenesis in ischemic muscle by stimulation of Toll-like receptor 3 signalling

Johannes Holfeld¹, Can Tepeköylü¹, Radoslaw Kozaryn¹, Karin Albrecht-Schgoer², Christoph Krapf¹, Rudolf Kirchmair², Kai Zacharowski³, Anja Urbschat⁴, Michael Grimm¹ and Patrick Paulus³

¹ Department of Cardiac Surgery, Innsbruck Medical University, Innsbruck, Austria

² Department of Internal Medicine I, Innsbruck Medical University, Innsbruck, Austria

³ Clinic of Anesthesiology, Intensive Care Medicine and Pain Therapy, Goethe-University Hospital Frankfurt, Frankfurt am Main, Germany

⁴ Clinic of Urology, Goethe-University Hospital Frankfurt am Main, Germany

Background: Low energy shock waves (SW) have been shown to induce angiogenesis in ischemic myocardium. The mechanism translating the physical stimulus to a biological signal is unknown. Toll-like receptor (TLR)-3 is activated by RNA binding. It plays a key role in inflammation and angiogenesis. We therefore hypothesized that SW cause cellular cavitation, thus liberating cytoplasmic mRNA that activates TLR-3 as does the specific agonist Poly I:C. Effects are suppressed in TLR-3 silenced cells and in TLR-3 knock out mice.

Methods: The effect of SW was tested in human umbilical vein endothelial cells (HUVECs): untreated (control) vs. SW treated (SW group) vs. treated with 200 µg/ml Poly I:C (agonist). TLR-3 gene silencing was done with siRNA. Hind limb ischemia was performed in wild type and TLR-3 knock-out mice. Expression of mRNA and proteins of the TLR-3 signaling pathway as well as typical angiogenic genes and proteins were measured. Laser Doppler perfusion imaging and necrosis score were assessed for clinical outcome evaluation (n=6).

Results: Shock wave treatment of HUVECs shows increase of mRNA expression (% of control) as does Poly I:C after 2 hours: TLR-3 (SW group 123.8 ± 8.0 and agonist group 237.7 ± 14.1, p<0.0001), Tie-2 (SW group 154.3 ± 20.0 and agonist 125.7 ± 12.3, p<0.008). TLR-3 gene silencing in SW treated HUVECs causes loss of response for TLR-3 mRNA (107.0 ± 13.3) as compared to SW group (378.3 ± 14.2) or agonist (1261 ± 72.1), both p<0.0001.

SW treated TLR-3 knock-out mice showed no improvement of perfusion ratio 4 weeks after hind limb ischemia (0.52 ± 0.07 vs. 0.53 ± 0.02 controls, p>0.05), whereas SW treated wild type animals improved significantly (0.78 ± 0.03 vs. 0.48 ± 0.08 controls, p=0.015). Pro-angiogenic genes and proteins were up-regulated significantly. All known TLR-3 signaling pathways were involved as shown by significant increase of key proteins Trif, TRAF6 and IRF3.

Conclusion: Low energy shock waves induce angiogenesis in ischemic muscle by stimulation of Toll-like receptor 3 signaling in endothelial cells. Effects are suppressed in TLR-3 silenced cells and in TLR-3 knock-out mice.